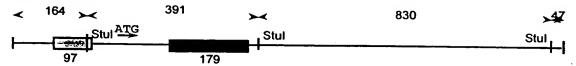
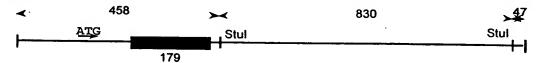


Figure 1

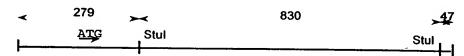
Full Length:



Properly Spliced:



Downstream Missplice:



Unsplice Intron and Downstream Missplice:



Upstream Splice Site (part of the expression vector)

'Weird' Splice Site (found in the Gcc cDNA sequence)

Figure 2

r rAG/ qua aqu Consensus Donor

5'- AAG CCG TTG AGT AGG/ GTA AGC ATC ATG GCT GGC AGC CTC AC 160a) unmodified Gcc

Lys Pro Leu Ser Arg Val Ser Ile Met Ala Gly Ser Leu Thr

- 5'- AAG CCG TTG AGT AGA GTC TCC ATC ATG GCT GGC AGC CTC AC
- b) " modifi4ed Gcc"

YYY YYY YNC aq/G Consensus Acceptor

5'- TTT CCT GCC CTT GGT ACC TTC AG/C CGC TAT GAG AGT ACA C 340a) unmodified Gcc

Phe Pro Ala Leu Gly Thr Phe Ser Arg Tyr Glu Ser Thr Arg 83

5' TTT CCT GCC CTG GGA ACA TTT TCC CGC TAT GAG AGT ACA C 340-

Figure 3 (continued on next page)

b) modified Gcc

Product of Alternatively Spliced mRNA transcript from the unmodified Gcc cDNA:

NOTE:

Nucleotides associated with RNA splice-sites are underlined. Those believed to be of most importance to splice site-recognition are double underlined.

" /" in Consensus Splice Sequence indicates splice site. " *"
under a particular nucleotide indicates a base changed in the
site-directed mutagenesis procedure.

r = C or A, y = c or u, and n = any.

Figure 3 (continued on next page)

Partial produce of the crypic splicing of Gcc cDNA

5'- AAG CCG TTG AGT AGG CCG CTA TGA GAG TAC AC

Lys Pro Leu Ser Arg16 Pro Leu STOP20!!

Figure 3 (continued)

	•			
10	20 30	40	50 60	
123456789012345	678901234567890	123456789012345	678901234567890	
NotI				
NGCGGCCGCTTAGCT	TGACTTAAGAAGGCC	GACGCCATGGAGTTT MetGluPhe	TCAAGTCCTTCCAGA SerSerProSerArg	
GAGGAATGTCCCAAG	CCTTTGAGTAGAGTC	TCCATCATGGCTGGC	AGCCTCACAGGTTTG	12
GluGluCysProLys	ProLeuSerArgVal	SerIleMetAlaGly	SerLeuThrGlyLeu	
CTTCTACTTCAGGCA	GTGTCGTGGGCATCA	GGTGCCCGCCCCTGC	ATCCCTAAAAGCTTC	18
LeuLeuLeuGlnAla	ValSerTrpAlaSer	GlyAlaArgProCys	IleProLysSerPhe	
GGCTACAGCTCGGTG	GTGTGTGTCTGCAAT	GCCACATACTGTGAC	TCCTTTGACCCCCCG	24
GlyTyrSerSerVal	ValCysValCysAsn	AlaThrTyrCysAsp	SerPheAspProPro	
ACCTTTCCTGCCCTG	GGAACATTTTCCCGC	TATGAGAGTACACGC	AGTGGGCGACGGATG	30
ThrPheProAlaLeu	GlyThrPheSerArg	TyrGluSerThrArg	SerGlyArgArgMet	
GAGCTGAGTATGGGG	CCCATCCAGGCTAAT	CACACGGGCACAGGC	CTGCTACTGACCCTG	36
GluLeuSerMetGly	ProlleGlnAlaAsn	HisThrGlyThrGly	LeuLeuLeuThrLeu	
CAGCCAGAACAGAAG	TTCCAGAAAGTGAAG	GGATTTGGAGGGGCC	ATGACAGATGCTGCT	42
GlnProGluGlnLys	PheGlnLysValLys	GlyPheGlyGlyAla	MetThrAspAlaAla	
GCTCTCAACATCCTT	GCCCTGTCACCCCCT	GCCCAAAATTTGCTA	CTTAAATCGTACTTC	48
AlaLeuAsnIleLeu	AlaLeuSerProPro	AlaGlnAsnLeuLeu	LeuLysSerTyrPhe	
TCTGAAGAAGGAATC (SerGluGluGlyIle	GGATATAACATCATC GlyTyrAsnIleIle	CGGGTACCCATGGCC ArgValProMetAla	AGCTGTGACTTCTCC SerCysAspPheSer	54
ATCCGCACCTACACC 1	TATGCAGACACCCCT TyrAlaAspThrPro	GATGATTTCCAGTTG AspAspPheGlnLeu	CACAACTTCAGCCTC HisAsnPheSerLeu	60
CCAGAGGAAGATACC A	AAGCTCAAGATACCC LysLeuLysIlePro	CTGATTCACCGAGCC LeulleHisArgAla	CTGCAGTTGGCCCAG LeuGlnLeuAlaGln	66
CGTCCCGTTTCACTC (ArgProValSerLeu I	LeuAlaSerProTrp	ThrSerProThrTrp	CTCAAGACCAATGGA LeuLysThrAsnGly	72

10	20 30	40	50 60	
123430783012343	6/090123456/890	123456789012345	678901234567890	
GCGGTGAATGGGAAG	GGGTCACTCAAGGGA	CAGCCCGGAGACATC	TACCACCAGACCTGG	780
AlavalAsnGlyLys	GlySerLeuLysGly	GlnProGlyAspIle	TACCACCAGACCTGG TyrHisGlnThrTrp	,00
GCCAGATACTTTGTG	AAGTTCCTGGATGCC	TATGCTGAGCACAAG	TTACAGTTCTGGGCA	940
AlaArgTyrPheVal	LysPheLeuAspAla	TyrAlaGluHisLys	LeuGlnPheTrpAla	840
			•	
GTGACAGCTGAAAAT	GAGCCTTCTGCTGGG	CTGTTGAGTGGATAC	CCCTTCCAGTGCCTG	200
ValThrAlaGluAsn	GluProSerAlaGly	LeuLeuSerGlyTyr	ProPheGlnCysLeu	900
GGCTTCACCCCTGAA	CATCAGCGAGACTTA	ATTGCCCGTGACCTA	CCTCCTACCCTCCT	
GlyPheThrProGlu	HisGlnArgAspLeu	IleAlaArgAspLeu	GlyProThrLeuAla	960
AACAGTACTCACCAC	AATGTCCGCCTACTC	ልጥርርጥርር አጥር አ ርር እ አ	CGCTTGCTGCTGCCC	
AsnSerThrHisHis	AsnValArgLeuLeu	MetLeuAspAspGln	Argleuleuleupro	1020
		2 2	gadaacaacaa to	
CACTGGGCAAAGGTG	GTACTGACAGACCCA	GAAGCAGCTAAATAT		
HisTrpAlaLysVal	ValLeuThrAspPro	GluAlaAlaLysTyr	ValHisGlyTleAla	1080
		, , , , , , , , , , , , , , , , , , , ,	· water of the state of the sta	
GTACATTGGTACCTG	GACTTTCTCCCTCCA	GCCAAAGCCACCCTA	66661 61 61 61 61 61 61 61 61 61 61 61 6	
ValHisTrpTyrLeu	AspPheLeuAlaPro	AlaLysAlaThrLeu	GlvGluThrHigAra	1140
			o-joraniminishig	
CTGTTCCCCAACACC	ATGCTCTTTCCCTCA	GAGGCCTGTGTGGGC	MCC3.3.5mmcmc	
LeuPheProAsnThr	MetLeuPheAlaSer	GluAlaCysValGly	SerlysPherroclu	1200
•		2	serbystmettpg10	
CAGAGTGTGCGGCTA	GGCTCCTCCCATCCA	GGGATGCAGTACAGC	C3 C3 CC	
GlnSerValArgLeu	GlySerTrpAspArq	GlyMetGlnTyrSer	UACAGCATCATCACG Hisserlellorba	1260
		1,	"TPDGLITGIII	
AACCTCCTGTACCAT	GTGGTCGCCTCGACC	CACIOCA A COmmo co		
AsnLeuLeuTyrHis	ValValGlyTrpThr	AspTrpAsnLeuAla	UTGAACCCCGAAGGA	1320
•			bearshirlogiagly	
GGACCCAATTGGGTG	ССТААСФФФСФССТАС	A CITCCCA III CA III III CITA	22 - 	
GlyProAsnTrpVal	ArgAsnPheValAsp	SerProIleIleVal	ASDILETBELYEAR	1380
	-			
ACGTTTTACAAACAG	CCCATGTTCTACCAC	ᢕᢔᠯᠮᢗᢗᢗᡃ᠋ᢗ᠘ᡎᡴᡊᡊ᠈ᠵᠵ	1 1 Compos mm =	
ThrPheTyrLysGln	ProMetPheTyrHis	LeuGlyHisPheSer	LVSPhelleProGlu	1440
Figure 4A(conti	nued on next pa	ge)	1	
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- 3° (

				
10	20 30	40	50	60
123456789012345 6	<u> 578901234567890</u>	123456789012345	678901234567	890
•				
CCCTCCCACACACTCC		63.63.63.65.6		
GGCTCCCAGAGAGTG G	Plut outral Na Com	CAGAAGAACGACCTG	GACGCAGTGGCA	TTG 1500
GlySerGlnArgVal G	stypenvatataser	GINLYSASNASPLeu	AspAlaValAla	Leu
ATGCATCCCGATGGC T	TCTGCTGTTGTGGTC	GTGCTAAACCGCTCC	ጥርጥል ልርርልጥናጥር	CCT 1560
MetHisProAspGly S	SerAlaValValVal	ValLeuAsnArgSer	SerLvsAspVal	Dro 1360
		3		110
CTTACCATCAAGGAT C	CTGCTGTGGGCTTC	CTGGAGACAATCTCA	CCTGGCTACTCC	ATT 1620
LeuThrIleLysAsp F	ProAravarGryPne	LeuGluThrIleSer	ProGlyTyrSer	Ile
CACACCTACCTGTGG C	CATCGCCAGTGATGG	AGCAGATACTCAAGG	ACCCACTCCCCT	CAC 1600
HisThrTyrLeuTrp H	HisArgGln		MODERCI GGGC I	CAG 1680
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC			No	
CCTGGGCATTAAAGG G	JACAGAGTCAGCGAA	TTCTGCAGATATCCA	TCACACTGGCGG	CCG 1740
			·	
С				1741
				1741

Figure 4A(continued)

10	20 30	40	50 60	
<u>123456789012345</u>	<u>678901234567890</u>	123456789012345	678901234567890	
NotI				
NGCGGCCGCTTAGCT	TGACTTAAGAAGGCC	GACGCCATGGAGTTT MetGluPhe	TCAAGTCCTTCCAGA SerSerProSerArg	60
GAGGAATGTCCCAAG	CCTTTGAGTAG <u>A</u> GT <u>C</u>	TCCATCATGGCTGGC	AGCCTCACAGGTTTG	120
GluGluCysProLys	ProLeuSerArgVal	SerlleMetAlaGly	SerLeuThrGlyLeu	
CTTCTACTTCAGGCA	GTGTCGTGGGCATCA	GGTGCCCGCCCCTGC	ATCCCTAAAAGCTTC	180
LeuLeuLeuGlnAla	ValSerTrpAlaSer	GlyAlaArgProCys	IleProLysSerPhe	
GGCTACAGCTCGGTG	GTGTGTGTCTGCAAT	GCCACATACTGTGAC	TCCTTTGACCCCCCG	240
GlyTyrSerSerVal	ValCysValCysAsn	AlaThrTyrCysAsp	SerPheAspProPro	
ACCTTTCCTGCCCT <u>G</u>	GGAACATT <u>TTC</u> CCGC	TATGAGAGTACACGC	AGTGGGCGACGGATG	300
ThrPheProAlaLeu	GlyThrPheSerArg	TyrGluSerThrArg	SerGlyArgArgMet	
GAGCTGAGTATGGGG	CCCATCCAGGCTAAT	CACACGGGCACAGGC	CTGCTACTGACCCTG	360
GluLeuSerMetGly	ProIleGlnAlaAsn	HisThrGlyThrGly	LeuLeuLeuThrLeu	
CAGCCAGAACAGAAG	TTCCAGAAAGTGAAG	GGATTTGGAGGGGCC	ATGACAGATGCTGCT	420
GlnProGluGlnLys	PheGlnLysValLys	GlyPheGlyGlyAla	MetThrAspAlaAla	
GCTCTCAACATCCTT	GCCCTGTCACCCCCT	GCCCAAAATTTGCTA	CTTAAATCGTACTTC	480
AlaLeuAsnIleLeu	AlaLeuSerProPro	AlaGlnAsnLeuLeu	LeuLysSerTyrPhe	
TCTGAAGAAGGAATC	GGATATAACATCATC	CGGGTACCCATGGCC	AGCTGTGACTTCTCC	540
SerGluGluGlyIle	GlyTyrAsnIleIle	ArgValProMetAla	SerCysAspPheSer	
ATCCGCACCTACACC	TATGCAGACACCCCT	GATGATTTCCAGTTG	CACAACTTCAGCCTC	600
IleArgThrTyrThr	TyrAlaAspThrPro	AspAspPheGlnLeu	HisAsnPheSerLeu	
CCAGAGGAAGATACC	AAGCTCAAGATACCC	CTGATTCACCGAGCC	CTGCAGTTGGCCCAG	660
ProGluGluAspThr	LysLeuLysIlePro	LeuIleHisArgAla	LeuGlnLeuAlaGln	
CGTCCCGTTTCACTC	CTTGCCAGCCCCTGG	ACATCACCCACTTGG	CTCAAGACCAATGGA	720
ArgProValSerLeu	LeuAlaSerProTrp	ThrSerProThrTrp	LeuLysThrAsnGly	

Mutations made to the Gcc insert are underlined

Figure 4B (continued on next page)

10 123456789012345	20 30 678901234567890	40	50 60	
	0,0001234307000	123430703012343	676901234567890	
CCCCMCA AMCCCA A C	CCCTC CTC CTC	•		
AlaValAsnGlvLvs	.GGGTCACTCAAGGGA	CAGCCCGGAGACATC	TACCACCAGACCTGG TyrHisGlnThrTrp	780
niavarnondry by 5	GIYSELLEUDYSGIY	GINFIOGIYASPITE	TyrnisGinThrTrp	
000101m10mmomo				
AlaArgTvrPheVal	AAGTTCCTGGATGCC	TATGCTGAGCACAAG	TTACAGTTCTGGGCA LeuGlnPheTrpAla	840
	nintrecedualita	TYTATAGTUNISLYS	renginhuelibala	
	G) GGGGGGGGGGG			
ValThrAlaGluAsn	GAGCCTTCTGCTGGG GluProSerAlaGly	CTGTTGAGTGGATAC	CCCTTCCAGTGCCTG ProPheGlnCysLeu	900
,	Grafioserriagry	negregaterative	Propriectorysten	
CCCMMC3 CCCCMC3 3	01mg10gg101			
GlvPheThrProGlu	HisGlnArgAspLeu	ATTGCCCGTGACCTA	GGTCCTACCCTCGCC GlyProThrLeuAla	960
		rrentunigaspheu	GIALLOLULFERATS	
እ <i>እርእርመ</i> እርመር እርር አር	3.3.0000000000000000000000000000000000	1000000000000000000000000000000000000		
AsnSerThrHisHis	AATGTCCGCCTACTC AsnValArgLeuLeu	ATGCTGGATGACCAA	CGCTTGCTGCTCCC	1020
		nechemaprapatii	vranementento	
CACTCCCCAAACCTC	CERCECTOR CROSS	01100100m11mm		
HisTrpAlaLysVal	ValLeuThrAspPro	GluAlaAlaLvsTvr	GTTCATGGCATTGCT ValHisGlyIleAla	1080
-	F		varnisdiyilenia	
CTACATTCCTACCTC	GACTTTCTGGCTCCA	CCC) A A CCCA CCCMA	60001010101010	
ValHisTrpTyrLeu	AspPheLeuAlaPro	AlaLysAlaThrLeu	GGGGAGACACCCGC GlyGluThrHisArg	1140
	_		o-journment	
CTGTTCCCCAACACC	ATGCTCTTTGCCTCA	GAGGCCTCTCTCTCCCC	TCCAAGTTCTGGGAG	1000
LeuPheProAsnThr	MetLeuPheAlaSer	GluAlaCysValGly	SerLysPheTrpGlu	1200
		_		
CAGAGTGTGCGGCTA	GGCTCCTGGGATCGA	GGGATGCAGTACACC	CACACCAMCAMCACC	1260
GlnSerValArgLeu	GlySerTrpAspArg	GlyMetGlnTyrSer	HisSerIleIleThr	1200
	·			
AACCTCCTGTACCAT	GTGGTCGGCTGGACC	GACTGGAACCTTGCC	CTGAACCCCGAAGGA	1320
AsnLeuLeuTyrHis	ValValGlyTrpThr	AspTrpAsnLeuAla	LeuAsnProGluGly	1320
				•
GGACCCAATTGGGTG	CGTAACTTTGTCGAC	AGTCCCATCATTGTA	GACATCACCAAGGAC	1380
GlyProAsnTrpVa1	ArgAsnPheValAsp	SerProIleIleVal	AspIleThrLysAsp	
ACGTTTTACAAACAG	CCCATGTTCTACCAC	CTTGGCCATTTCAGC	AAGTTCATTCCTGAG	1440
TorPheTyrLysGln	ProMetPheTyrHis	LeuGlyHisPheSer	LysPheIleProGlu	
			•	

. Mutations made to the Gcc insert are underlined

Figure 4B (continued on next page)

10	20 30	40	50 60	
123456789012345	678901234567890	123456789012345	678901234567890	
GGCTCCCAGAGAGTG	CCCCTCCCTTCCCACT	C1C11C11CC1CCTC		
GlySerGlnAraVal	GlyLeuValAlaSer	CAGAAGAACGACCTG	GACGCAGTGGCATTG	1500
0-10010111119141	Olybedvalklasel	GIILLYSASHASPLEU	AspatavatAtaLeu	
ATGCATCCCGATGGC	TCTGCTGTTGTGGTC	GTGCTAAACCGCTCC	TCTAAGGATGTGCCT	1560
MetHisProAspGly	SerAlaValValVal	ValLeuAsnArgSer	SerLysAspValPro	1300
СТТАССАТСААССАТ	CCTGCTGTGGGCTTC	CMCC3C3C33mcmc3	CCTCCCTT	
LeuThrIleLvsAsp	ProAlaValGlyPhe	LeuGluThrIloCor	CCTGGCTACTCCATT	1620
		pedetatifftteset	Progryfyrserite	٠
CACACCTACCTGTGG		AGCAGATACTCAAGG	AGGCACTGGGCTCAG	1680
HisThrTyrLeuTrp	HisArgGln	•		
			NotI	
CCTGGGCATTAAAGG	GACAGAGTCAGCGAA	ጥጥርጥርር እር አ ጥአጥር ር አ		2740
		TICIOCAGATATCCA	ICACACIGGCGGCCG	1740
С				1741

Mutations made to the Gcc insert are underlined

Figure 4B (continued)

10	20 30	40	50 60	
123430707012343	070301234367890	123456789012345	678901234567890	<u> </u>
GGCTCCCAGAGAGTG GlySerGlnArgVal	GGGCTGGTTGCCAGT GlyLeuValAlaSer	CAGAAGAACGACCTG GlnLysAsnAspLeu	GACGCAGTGGCATTG AspAlaValAlaLeu	1500
ATGCATCCCGATGGC	TCTGCTGTTGTGGTC	GTGCTAAACCGCTCC	TCTAAGGATGTGCCT	1560
MetHisProAspGly	SerAlaValValVal	ValLeuAsnArgSer	SerLysAspValPro	
CTTACCATCAAGGAT LeuThrlleLysAsp	CCTGCTGTGGGCTTC ProAlaValGlyPhe	CTGGAGACAATCTCA LeuGluThrIleSer	CCTGGCTACTCCATT ProGlyTyrSerIle	1620
CACACCTACCTGTGG	CATCGCCAGTGATGG	AGCAGATACTCAAGG	AGGCACTGGGCTCAG	1680
HisThrTyrLeuTrp	HisArgGln			
ССПССССВ ФТВ В В СС	CACACACMCACCOA	mmamaaa aa	Noti	
CC1000CATTAAAGG	GACAGAGTCAGCGAA	TTCTGCAGATATCCA	TCACACTGGCGGCCG	1740
С				1741

Mutations made to the Gcc insert are underlined

Figure 4B (continued)